

**AMINO ACID COMPOSITION, FATTY ACID PROFILE AND CHARACTERIZATION OF
VEGETABLE OIL FROM *HURA CREPITANS* SEED**

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ABSTRACT

There is a great need for evaluation of nutritional component and quality of emerging functional food oil to provide scientific data to support their possible beneficial health effect. The present study was designed to evaluate the nutritional quality parameters of oil extracted from *Hura crepitans* seed. The oil was analyzed for physicochemical properties, fatty acid profile and amino acid profile using standard methods. The percentage composition of essential amino acid ranges from 1.39 to 0.21g/100g of oil, while that of non-essential amino acid ranges from 1.32 to 0.3g/100g. However, the concentration of arachidonic acid (281.8 µg/dl) and ricinoleic acid (231.4 µg/dl) were found to be the most abundant in fatty acids. Linoleic and linolenic acids are unsaturated fatty acids were also found at 187.7 and 148.6 µg/dl levels of concentration, respectively. Dietary consumption of *Hura crepitans* seed oil may promote health status by providing nutrients such as essential amino acids and fatty acids.

Keywords: *Hura crepitans*, amino acid, fatty acid, vegetable oil, seeds

INTRODUCTION

The desire to conserve resources spent on importation of oil for domestic and industrial use has given a fresh impetus in the search for novel sources to complement the traditional ones (Aremu *et al.*, 2006). There has been a

focus on non-utilized oil seeds for possible development and use (Obasi and Okoli, 1993) as these seeds are wasted from season to season. Attention has therefore, been shifted to under-utilized local seeds for possible

development and use as there are several of these under-exploited plant seeds in Nigeria. Seeds have nutritive and calorific values which make them relevant in diets. They are also good sources of edible oils (Odoemelan, 2005), but a whole lot of seeds have not been exploited at all due to certain repulsive contents.

Seeds are good sources of oil needed for cooking, cake meal and margarine, and are valuable protein supplement for all farm animals. Seeds are also of value in the production of confectionaries such as cheese, sweets, crisps, sandwiches, cookies and for flavouring foods. They can be roasted, fried or boiled and eaten as snacks. Seed flours are incorporated into infant foods such as root and tuber based diets, which are low in protein in order to increase their protein content and improve flavor. Oil obtained from these unexploited plant seeds are essential in meeting global nutritional demands and are utilized for many food and other industrial purposes (Idouraine *et al.*, 1996).

In view of the economic situation all over the world and the high level of oil consumption among the populace, it is essential to source for inexpensive and good quality seeds and other oil bearing plant seeds (Ayoade, 2015). Seeds can supply essential nutrients such as amino acids and essential fatty acids, (especially for *Azania africana* and *Deutarium microcrpum*) that the body cannot synthesize, but must be

supplied exogenously. It showed also that seeds can maintain the protein/nitrogen balance in the body in amount comparable to standard protein sources (Igwenyi and Akubugwo, 2010).

The discovery of new sources of plant proteins from seeds will help to supplement the nutrients of staple carbohydrate foods. The ultimate success of utilizing plant proteins as food ingredients largely depends upon the beneficial qualities they impact to food which in turn depend on their nutritional and functional properties (Afolabi *et al.*, 1984). Africa and indeed most tropical and subtropical countries are blessed with numerous seeds and fruits. Many of which are yet to be fully exploited due to a dearth of information on their chemical, biological and industrial importance.

Hura crepitans Linn is a tropical plant belonging to the family Euphorbiaceae called "Monkey no climb". In Nigeria, it is known as 'Odan Mecca' by the Kabba people of Kogi State and "Aroyin" by the Ijesha people of Osun State and "Osisi ogwu, meaning spiny tree in Igbo speaking States of Nigeria. It is often cultivated for shade. Fishermen use the milky, caustics from this tree to poison fish. The Caribs made arrow poison from its sap. The wood is used for furniture under the name "hura" (Jones, 2007). Before modern pens were invented, the unripe seed pods of *Hura crepitans* were sawed in half to make

decorative pen sandboxes, hence the name 'sandbox tree'. It bears flowers usually at the beginning and at the end of rainy season. Consumption of the seeds gives a burning sensation in the throat, induce vomiting and purging, suffocating and headache (Allen, 2000). It has been further stressed that people who had eaten the shells with the seeds were seized with violent vomiting and headache while those who ate the seeds without the shell suffered from nausea and violent pain in stomach, vomited once and had violent diarrhea (Allen, 2000). A milky juice that is

present in all parts of the plant can cause blindness if applied to the eye, and oil of *Hura crepitans* can be used as a purgative (Fowomola and Akindahunsa, 2005), elephantiasis and leprosy (Allen, 2000). Despite all these negative findings against *Hura crepitans* seed, the food potential of *H. crepitans* seed has not been investigated. However, competitions for commercial edible plant oils which are obtainable from species of plants have necessitated the search for oils from underutilized tropical plants (Mestrallet *et al.*, 2004).



Figure 1: *Hura crepitans* fruit



Figure 2: *Hura crepitans* seeds

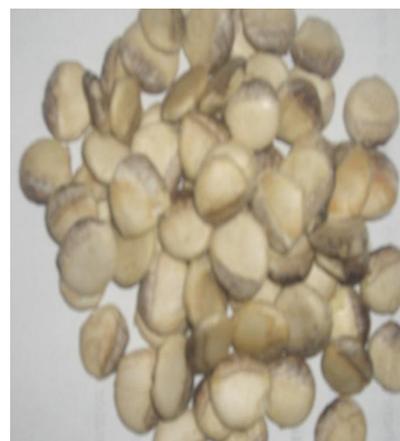


Figure 3: *Hura crepitans* kernel



Figure 4: *Hura crepitans* dry fruit.



Figure 5: Dehisced fruit and seeds.

The potential of this plant has remained untapped. The present study, therefore, aimed at investigating the chemical composition and physicochemical properties of vegetable oil

produced from sandbox (*Hura crepitans*) seed in order to give adequate information on its suitability for possible nutritional and industrial applications. The plant has not been recorded

as having any nutritional property and has been planted basically for ornamental purposes. This work is an attempt to discover potential nutritional endowment by investigation of the amino acid profile, fatty acid composition and physicochemical characteristics of vegetable oils of *Hura crepitans* seeds produced in Nigeria.

The results obtained may provide guidelines for industrialists and manufacturers who are seeking for varieties of plants with high oil yield and unique properties for industrial applications. Amino acid profile, fatty acid composition and physicochemical characteristics of this oil is necessary for its potentials and as a subject of investigation to increase knowledge especially when comparing values of the oil from different plant sources and sampling locations (Akbar *et al.*, 2009).

MATERIALS AND METHODS

Biological sample used in this work is dry seeds of *Hura Crepitans* plant. It was collected from a *Hura Creptans* tree at F-41, Democracy Estate, Abakaliki, Ebonyi State, Nigeria, where the it was planted as an ornamental and shade plant. Seeds of *Hura creptans* were decorticate, milled with a electronic blender and homogenized to a very fine flour. The resulting flour was used for the study.

Determination of Fatty Acid Profile (AOAC, 1980): The determination of chain length distribution of fatty acids methyl esters was carried out using the method number SO29P

packed GC (PORIM, 1999). The fatty acid methyl esters of lipids were prepared according to the AOAC method 991.39 (AOAC, 1984). The analyses of fatty acid methyl esters were carried out with a Hewlett Packard Gas Chromatograph equipped with a hydrogen flame ionization detector and a capillary column; CP-Sil-88 Wcott fused silica 50mx0.25mm id.(of 0.20mm film thickness). The temperatures of injector and detector were 270⁰C. The initial column temperature was 170⁰C and and gradually increased to 205⁰C at a rate of 1⁰C/min. The split ratio was 1/50. The carrier gas was hydrogen at a flow rate of 1ml/min. The identification and quantification of fatty acid methyl ester was accomplished by comparing retention times of the peaks with those of standards.

Determiation of Amino Acid Profile: The amino acid profile of the seeds was determined using amino acid analyzer, technicon TSM-1 (model: DNA 0209) and methods described by Spackman *et al.*, (1985). The known samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyzer (TSM).

Defatting the Sample: A known weight of the dried sample was weighed into extraction thimble and the fat extracted using chloroform/methanol (ratio 2:1 mixture) using soxhelet extraction apparatus as described by

AOAC (2006). The extraction lasted for 15 hours.

Nitrogen Determination: The nitrogen content was determined using the Kjeldhal method. The hydrolyzed sample was into the TSM analyzer: The amount loaded was between 5-10 microliter. This was dispensed into the cartridge of the analyzer. The TSM is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for about 76 minutes. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height was accurately measured and recorded. Approximate area of each peak was then obtained by triangulation by multiplying the height with half of the width (1/2 base x height). The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated.

Oil extraction fat/oil content (AOAC, 1980):

The soxhlet apparatus method was employed

for oil extraction using petroleum ether (40-60⁰C). Specific gravity was calculated as the ratio of the apparent mass, determined by weight in air of a given volume of the oil at t⁰C to that of the same volume of water at 20⁰C whereas the apparent density (at t⁰C) is the apparent mass in grams, determined by weighing in air, 1ml of the oil at t⁰C (Apple, 1980). The acid value was determined using Standard Official Methods (AOAC, 1980). The wheeler method as described by Hamilton *et al.*, (1992) was used in the determination of the peroxide value. The Wij's method as described by Hendriske and Harwood (1986) was used in determining the iodine value. The saponification value and unsaponifiable matter were determined using standard methods (AOAC, 1980).

RESULTS

Table 1 shows the amino acid profile of seed oil of *Hura crepitans*. It was observed that the seed oil contains 10 essential amino acids and 10 non-essential amino acids.

Table 1: Amino Acid Profile of *Hura crepitans* seed oil

S/N	Amino Acids	Percentage Composition (%)
1	*Arginine	1.04
2	*Histidine	0.21
3	*Isoleucine	0.64
4	*Leucine	1.39
5	*Lysine	1.03
6	*Methionine	0.37
7	*Phenylalanine	0.68
8	*Threonine	0.97
9	*Tryptophan	0.83
10	*Valine	1.94
11	Alanine	0.41
12	Asparagine	1.32
13	Aspartatic acid	0.31
14	Trimethyl serine	0.46
15	Glutamic acid	0.30
16	Glutamine	0.40
17	Glycine	1.02
18	Proline	0.31
19	Serine	0.57
20	Tyrosine	0.37

* Essential amino acids.

Table 2: Fatty Acid Profile of *Hura crepitans* Seeds

S/N	Fatty Acids	Concentration (µg/dl)
1	Palmitic acid	196.156
2	Oleic acid	175.204
3	Linoleic acid	187.651
4	Linolenic acid	148.619
5	Recinoleic acid	231.370
6	Arachidonic acid	281.762

The results showed that the oil contains both essential and non-essential fatty acids.

Table 3: Physicochemical characterization of extracted oil of *Hura crepitans* seed

S/N	Physicochemical parameter	Concentration (µg/dl)
1	% Moisture	0.62 ± 0.0002%
2	Specific gravity	1.1086 ± 0.025
3	Acid value	4.488mg ± 0.240KOH/g
4	Peroxide value	0.560 ± 0.002
5	Saponification value	185.13mg ± 2.78KOH/g
6	Iodine value	47.299 ± 1.64g/100g

DISCUSSION

The present study reveals the presence of extractable oil and essential nutrients in the seed oil of *Hura crepitans*. The percentage composition of the essential amino acids were in the order valine > leucine > arginine > lysine > threonine > tryptophan > phenylalanine > isoleucine > methionine > histidine with the values of 1.39, 1.39, 1.04, 1.03, 0.97, 0.83, 0.68, 0.64, 0.37 and 0.21g/100g protein respectively. The percentage compositions of the nonessential amino acids were in the order: asparagines > glycine > serine > trimethyl serine > alanine > glutamine > tyrosine > aspartic acid > proline > glutamic acid corresponding to the values of 1.32, 1.02, 0.57, 0.46, 0.41, 0.40, 0.37, 0.31, 0.31 and 0.30g/100g protein respectively.

The more abundant amino acids were valine, leucine, asparagine, arginine, and glycine with the values of 1.94, 1.39, 1.32, 1.04 g/100g protein, respectively. This is an indication that *Hura crepitans* seed flour can be a good source

of some essential amino acids of protein. Robust body of evidence indicates that histidine is an essential amino acid for adults; its dietary requirement in both normal and uremic men has been reported to be between 8 and 12 mg/kg/day (Giordano *et al.*, 1972). In comparison, it was observed that our results in the current study is lower than the values reported by Olatidoye *et al.*, (2010) in which the values of glutamic acid, arginine, aspartic acid and leucine were 15.2, 5.8, 5.2 and 5.1 g/100g protein, respectively. However, this observation is in close agreement with the work of Olaofe *et al.*, (1994) and Oshodi *et al.*, (1998) reporting glutamine content in some of the Nigerian legumes; lima bean (7.45g/100g), pigeon pea (8.40g/100g) and Africa yam (7.45g/100g). The seeds *Hura crepitans* were found to be rich in essential amino acids which are necessary as precursors of many other amino acids and vital cellular substances such as hormones and important proteins. A poor diet can have an injurious impact on health,

causing deficiency diseases such as scurvy, beriberi, and kwashiorkor; health threatening conditions like obesity and metabolic syndrome, and such common chronic systemic diseases as cardiovascular disease, diabetes, and osteoporosis (Igwenyi and Akubugwo, 2010).

The result on fatty acid profile (Table 2) showed that the level of arachidonic acid and ricinoleic acid from the results were found to be 281.8 and 231.4 $\mu\text{g}/\text{dl}$ respectively which revealed that the two fatty acids were the most abundant fatty acids in the sample. This order was reported to be the case with most plant seed oils (Siddhuraju *et al.*, 2001). Other fatty acids like palmitic acid, linoleic acid, oleic acid and linolenic acid found in this investigation were present in measurable quantities in the sample. It was observed that the concentrations of all the fatty acids in this study are much higher than the values reported for *Hura crepitans* by Oyekeke (2012) and Alabi *et al.*, (2013). This may be due to variation in season, soil nutrient, humidity, temperature and environmental features. Fatty acids serve as the building blocks of fat in the body and in food and are source of energy as well as constituents of cellular membranes. They play important roles in nourishing and protecting babies from infections. Linoleic and linolenic acids are unsaturated fatty acids and their deficiencies can cause loss of hair, susceptibility to infection, poor wound heal and

circulatory problem (Oyekunle, 2007). These essential fatty acids are important in maintaining cell membrane structure and in capillary wall integrity and serve as starting materials for the synthesis of other unsaturated fatty acid derivatives such as prostaglandins (Igwenyi, 2008).

The physicochemical properties of *H. crepitans* seed oil are shown in Table 3. The percentage moisture of the oil was found to be 0.62%. This value is lower than the value ($3.00 \pm 0.13\%$) obtained in the work reported by Oderinde *et al.*, (2009) on *Hura crepitans* seed. The specific gravity of the *H. crepitans* oil (1.1086 ± 0.025) was comparable with those obtained in a similar study by Olatidoye *et al.*, (2010) and Okolie *et al.*, (2012). This showed that the oil is less thick when compared with most drying oils whose refractive indices were between 1.475 and 1.485 (Duel, 1951). The oil could be used as lubricant in engine parts in the tropics if left overnight as solidification temperature of the oil is below 10°C at any season (Oyekunle *et al.*, 2007).

The acid value of the oil was high ($4.488\text{mg} \pm 0.24\text{KOH}/\text{g}$) although it was lower than the $19.04 \pm 0.41\text{mgKOH}/\text{g}$ and $28.05 \pm 2.30\text{mgKOH}/\text{g}$ reported by Oderinde *et al.*, (2009) and Okolie *et al.*, (2012). The acid value is expected to range from 0.00-3.00mgKOH/g oil before it can find application in cooking (Oyekunle *et al.*, 2007). But the value is high for oil under study. The acid value can be

reduced by subjecting the oil to refining and this may also improve its quality for industrial purposes. The peroxide value of *H. crepitans* oil in this study was 0.560 ± 0.002 mg/g oil. This value is lower than that expected of rancid oil which ranges from 20.00 to 40.00 mg/g oil (Oderinde *et al.*, 1998). This value compares favorably with commercial vegetable oils, suggesting that *H. crepitans* oil is likely to be slow to oxidative rancidity (Ajayi *et al.*, 2002). The concentration in $\mu\text{g}/\text{dl}$ of fatty acids was in the order arachidonic acid (281.762), ricinoleic acid (231.370), palmitic acid (196.156), linoleic acid (187.651), oleic acid (175.204) and linolenic acid (148.619). The significance of having these fatty acids makes it a potential raw material for the coating industry.

The saponification value of the oil was $185.13\text{mg} \pm 2.78\text{KOH}/\text{g}$ which is within the values obtained for some vegetable oils ranging from 188-196mg KOH/g (Oyekunle *et al.*, 2007). However, there are some vegetable oils with higher saponification values such as coconut oil (253 mgKOH/g), palm kernel oil (247mgKOH/g) and butter fat 225 mgKOH/g (Calder, 2004). It has been reported by Mestrallet *et al.*; (2004) that oils with higher saponification values contain high proportion of low molecular weight fatty acids. Therefore, the value obtained for *Hura crepitans* seed indicated that the oil contained high proportion of low molecular weight fatty acids. The saponification value is high and this suggests

possible use of the oil in production of liquid soap, shampoos and lather shaving creams. This observation is in close conformity to the findings of Ajayi *et al.*, (2002). Iodine value is a measure of the degree of unsaturation in an oil and it is an identity characteristic of native oil. It indicates the degree of unsaturation in the fatty acids of triacylglycerol. This value could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. The iodine value obtained is low which suggest the presence of saturated fatty acid and this places the oil in the non-drying groups. This oil may find application as a raw material in industries for the manufacture of vegetable oil-based ice cream (Oyekunle *et al.*, 2007).

CONCLUSION

The properties of the oil indicated that if the oil from *H. crepitans* seed is subjected to further refining, it could be useful in deep frying, emulsifier formulation, baking activities and as part of short cake and confectionary component as is a non-drying oil. It may also find application industries for manufacture of vegetable oil based ice cream, liquid soap, shampoos and shaving cream. The oil could also serve as grease for engine parts without causing sticking problems both in the tropics and temperate region as the oil is free flowing at these temperatures. It is also envisaged as a good potential base material for oil paint alkyd resins. The richness in protein as indicated by

the measure of essential and non-essential amino acids gives impetus in its consideration as a source of nutritional protein. However, future studies are encouraged to determine the presence of toxins and antinutrient in the seed.

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